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CD8$^+$/perforin$^+$/WC1$^-$ γδ T cells, not CD8$^+$ αβ T cells, infiltrate vasculitis lesions of American bison (*Bison bison*) with experimental sheep-associated malignant catarrhal fever

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ABSTRACT

Sheep-associated malignant catarrhal fever (SA-MCF) caused by ovine herpesvirus-2 (OvHV-2), a γ-herpesvirus in the *Macavirus* genus, is a fatal disease associated with lymphoproliferation, lymphocytic vasculitis, and mucosal ulceration in clinically susceptible species. SA-MCF is an important threat to American bison (*Bison bison*) due to their high susceptibility to this disease. Currently, the pathogenesis of disease in SA-MCF is poorly understood, and the immunophenotype of lymphocytes that infiltrate the vascular lesions of bison and cattle with SA-MCF has been only partially defined. Previous single-color immunohistochemistry studies have demonstrated that CD8$^+$ cells and CD4$^+$ cells predominate within vascular infiltrates in cattle and bison. The CD8$^+$ cells detected in the vascular lesions of cattle and bison were assumed to be cytotoxic αβ T lymphocytes. However, polychromatic immunophenotyping analyses in this study showed that CD8$^+$/perforin$^+$ γδ T cells, CD4$^+$/perforin$^-$ αβ T cells, and B cells infiltrate vascular lesions in the urinary bladder, kidney, and liver of six bison with experimentally-induced SA-MCF. CD8$^+$ αβ T cells and WC1$^+$ γδ T cell cells were only infrequently and inconsistently identified. This study confirmed our hypothesis that the predominant CD8$^+$ lymphocytes infiltrating the vascular lesions of bison with SA-MCF are cytotoxic lymphocytes of the innate immune system, not CD8$^+$ αβ T cells. Results of the present study support the previous suggestions that MCF is fundamentally a disease of immune dysregulation.

1. Introduction

Sheep-associated malignant catarrhal fever (SA-MCF) is an important ruminant disease with a worldwide distribution. The causative virus, ovine herpesvirus-2 (OvHV-2), is a γ-herpesvirus in the *Macavirus* genus. Infection is endemic, persistent, and subclinical in domestic and wild sheep, but triggers a severe and generally fatal systemic disease in clinically susceptible hosts such as cattle, deer, and bison. American bison (*Bison bison*) are particularly susceptible to disease, and outbreaks have occurred in American bison herds adjacent to sheep (Berezowski et al., 2005; Li, 2006; Li et al., 2008). In contrast, European cattle breeds (*Bos taurus*) are relatively resistant, and disease is more sporadic

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(Plowright, 1990; Crawford et al., 1998; Li, 2006). Currently, the pathogenesis of disease in SA-MCF is very poorly understood but appears to have similarities with other lymphotropic γ-herpesviruses including Epstein-Barr virus (EBV) and murine herpesvirus-68 (MHV-68).

Gross lesions of SA-MCF include corneal opacity, mucosal ulcerations and ecchymoses, and oculonasal discharge. The primary histological changes are lymphoproliferation, mucosal necrosis, and systemic vasculitis with perivascular and intramural accumulation of large lymphocytes. Vasculitis is limited to small- to medium-caliber arteries and veins, particularly in the urinary bladder, brain, liver, and kidney (Liggitt, 1980; Schultheiss et al., 1998, 2000).

The immunophenotype of lymphocytes that infiltrate the vascular lesions of bison and cattle with SA-MCF has been only partially defined. Previous single-color immunohistochemistry studies have demonstrated that $CD8^+$ cells and $CD4^+$ cells predominate within vascular infiltrates in cattle (Ellis et al., 1992; Nakajima et al., 1994; Lagourette et al., 1997), and $CD8^+$ cells and $CD3^+$ cells predominate within vascular lesions in the brains of bison (Simon et al., 2003). The $CD8^+$ cells detected in the vascular lesions of cattle and bison were previously assumed to be cytotoxic αβ T lymphocytes. However, CD8 is expressed on not only on bovine αβ T lymphocytes but also on γδ T cells and NK cells (Davis et al., 1996; Storset, 2004; Endsley, 2006; Weiss, 2006) suggesting that the $CD8^+$ cells could be lymphocytes of the innate immune system.

Previous phenotypic analyses of lymphocytes extracted from lymphoid tissues of cattle also suggest infected $CD8^+$ cells are cytotoxic lymphocytes of the innate immune system. These studies revealed infected lymphocytes expressed CD2, an adhesion molecule and activation receptor common to most lymphocytes, and CD25, a marker for activated αβ and γδ T lymphocytes and NK cells (Reid et al., 1989; Burrells and Reid, 1991; Schock et al., 1998). These lymphocytes also required exogenous IL-2 for culture (Schock et al., 1998) and developed MHC-unrestricted cytotoxicity (Cook and Splitter, 1988; Reid et al., 1989). Altogether, these observations led us to hypothesize that the $CD8^+$ cells infiltrating the vasculitis lesions of bison with clinical SA-MCF were cytotoxic lymphocytes of the innate immune system.

In order to more precisely identify the lymphoid cell types, we used newly available bovine immune cell markers and evaluated co-expression of multiple cell markers on lymphocytes infiltrating vascular lesions of bison with SA-MCF. Our results support the hypothesis that the predominant $CD8^+$ lymphocytes in the vascular lesions are cytotoxic γδ T cells, not $CD8^+$ αβ T cells. These findings provide a foundation for defining the role of the innate immune system in the pathogenesis of vasculitis in bison with SA-MCF.

2. Materials and methods

2.1. Research animals and samples

OvHV-2 uninfected, captive bison from Wyoming were identified by screening for antibody against the conserved MCF virus group 15A epitope with competitive inhibition ELISA and for OvHV-2 DNA by semi-nested PCR (Li et al., 1995, 2001). Persistent bovine viral diarrhea virus infection was ruled out by reverse transcriptase PCR (RT-PCR) and serology as previously described (Horner et al., 1995; Kramps et al., 1999). Six 1-year old, male bison were infected by nasal aerosolization with 2 mL of pooled sheep nasal secretions containing 1×10^7 OvHV-2 DNA copies collected from shedding sheep as described (Taus et al., 2005). This dose from the current inoculant pool reproducibly caused fatal disease in bison (O'Toole et al., 2007). Three uninfected 1-year old, male bison were used as controls: one was uninfected, and two were sham inoculated by nasal aerosolization with pooled nasal secretions from uninfected sheep. Clinical signs were monitored daily, and real-time PCR was used to measure OvHV-2 copies in peripheral blood lymphocytes (PBL) (Hussy et al., 2001).

Previous intra-nasal inoculation studies (O'Toole et al., 2007) and outbreaks of natural disease in bison (Schultheiss et al., 1998, 2000) showed that the clinical period is very short and bison commonly die prior to the onset of clinical signs. In our study we planned to evaluate vascular lesions within this short clinical period. Therefore, once OvHV-2 was detected by PCR in the blood and some bison began to develop clinical signs, animals were euthanized over a 10-day period. Full necropsies with histopathology were performed. Tissue samples including urinary bladder, kidney, and liver were collected and fixed in formalin or flash-frozen in Tissue-Tek® Optimal Cutting Temperature compound (Miles Inc., Elkart, IN). The experiments were approved and conducted according to Institutional Animal Care and Use Committee protocols.

2.2. Demonstration that bovine cell markers cross-react with bison tissues

Indirect immunofluorescence assays (IFA) were performed to verify that antibodies reactive with bovine leukocyte differentiation molecules recognize epitopes on homologous bison molecules. Flash-frozen samples of mesenteric lymph nodes and ileum from control bison and a splenectomized Holstein calf (*B. taurus*) were sectioned at 5 μm with a cryostat, mounted on charged slides, and fixed in 100% ethanol. For immunostaining, sections were rehydrated in blocker solution comprised of 10–15% goat serum/PBS/0.05% Tween® 20 (Fischer Scientific, Fair Lawn, NJ). The tissues were labeled with one or more of the primary antibodies listed in Table 1 for 60 min, subjected to several washing cycles, and then incubated for 30 min with AlexaFluor fluorochrome-conjugated isotype-specific goat anti-mouse or anti-rabbit secondary antibodies (Invitrogen Corporation, Carlsbad, CA) at 1 μg/mL. Sections were rinsed, cover-slipped using Molecular Probes® SlowFade Gold with DAPI (Invitrogen Corporation, Carlsbad, CA) and viewed with a Zeiss Axioskop 2 Plus fluorescent microscope (Carl Zeiss MicroImaging, Inc., Thornwood, NY). Replicate sections incubated with primary isotype- and concentration-matched antibodies generated from the same species, but specific for an irrelevant antigen, were used as negative controls with each assay. Murine negative control antibodies AV64 (IgG1), AV213 (IgG2a),

Table 1
Specific antibodies verified to be cross-reactive for cell marker identification in cattle and bison.

Cell subset(s)	Primary antibodies	References
αβ T cells	CD3ε chain (MM1A)	MacHugh et al. (1998)
	CD4 (ILA11.A)	Baldwin et al. (1986)
αβ or γδ T cells	CD8 (CACT80C)	MacHugh (1991); Galeotti et al. (1993)
	CD2 (BAQ95A)	Gutierrez et al. (1999)
	CD25 (GB112A)	Parsons (1996); Trueblood et al. (1998); Davis (2001)
γδ T cells	TCR1-N24 δ chain (GB21A)	MacHugh et al. (1997)
	WC1 (ILA29)	Clevers (1990)
NK cells	NKp46/CD335 (AKS1)	Storset (2004)
B cells	CD79a (HM57)[a]	Wangoo et al. (2005)
Monocytes/macrophages	M-M7 (BAQ151A)	Sopp et al. (1996)
	Calprotectin (MAC387)[a]	Gutierrez et al. (1999)
Vascular endothelium	Von Willebrand's factor (A0082)	Warren and Summers (2007)
	Von Willebrand's factor (F8/86)[b]	Buser et al. (2006); Warren and Summers (2007)
Perforin	Perforin (δG9)[c]	Endsley et al. (2004); Endsley et al. (2007)

All listed antibodies specific for cattle cell markers are effective in ethanol-fixed cryosections of bison lymphoid tissues and were used at 2–15 μg/mL concentrations. Unless otherwise indicated, antibodies were provided by William Davis, Monoclonal Antibody Center, Washington State University.

[a] AbD Serotec, Raleigh, NC.
[b] Dako North America, Inc., Carpinteria, CA.
[c] Santa Cruz Biotechnology, Inc., Santa Cruz, CA.

and GDBMB4 (IgG2b) were provided by the Monoclonal Antibody Center, Pullman, WA. The rabbit immunoglobulin fraction negative control was purchased from Dako North America, Inc., Carpinteria, CA. For each antibody, the staining pattern and intensity were compared between ethanol-fixed cryosections of cattle and bison mesenteric lymph node and ileum.

2.3. Immunophenotyping lymphocytes in vascular lesions

Leukocyte immunophenotyping was performed by polychromatic indirect immunofluorescent detection of leukocyte differentiation molecules on ethanol-fixed cryosections of urinary bladder, kidney, and liver. The source of all antibodies is noted in Table 1. Preliminary data were collected with a Zeiss Akioskop 2 Plus fluorescent microscope. Three-, four-, and five-color scans were collected with a Zeiss LCM 510 META laser scanning confocal microscope (Carl Zeiss MicroImaging, Inc., Jena, Germany) using the multi-track feature. Images for control slides and experimental slides were scanned at either the same or maximal exposure settings. Fluorophores and filters were chosen for adequate emission resolution, and the laser wavelengths utilized corresponded with the absorption maxima of each fluorophore. Appropriate pseudocolors were chosen for maximal visual detection and resolution (Gunnes et al., 2004). The emitted signal was recorded in three to four separate monochrome digital images, one for each fluorophore-labeled cell marker, and the imaging software merged these separate images into single images. Cells were counted using Cell Counter, version 07/01/2009 (www.rsbweb.nih.gov/ij/plugins/cell-counter.html), a plug-in for ImageJ, version 1.42q (NIH, USA, www.rsbweb.nih.gov/ij/). Statistical analyses were performed using GraphPad Instat v. 3.10 (GraphPad Software Inc., www.graphpad.com).

Vascular lesions in the urinary bladder of two bison with SA-MCF were localized using DAPI, a fluorescent nuclear stain identifying cell nuclei, and polyclonal antibodies specific for von Willebrand's factor (vWF), an endothelial cell marker. In addition, single leukocyte markers for CD2, CD3, CD4, CD8α, CD25, CD79a, CD335/NKp46, WC1, M-M7, calprotectin, and the TCR1-N24 δ chain (Table 1) were used to determine which of these markers were expressed by immune cells in the vascular lesions. Replicate sections incubated with primary isotype-matched and concentration-matched antibodies generated from the same species but specific for an irrelevant antigen were used as negative controls with each assay.

Lymphocytes were further phenotyped by concurrent immunofluorescent detection of three lymphoid markers in vascular lesions in the urinary bladder of six bison with SA-MCF and the kidney and liver of a single bison that had vascular lesions in all of these tissues. Phenotyping was based on currently established leukocyte differentiation molecule expression profiles of bovine immune cells for identification of bison lymphoid cell subsets (Table 1). Polychromatic immunofluorescence studies included the following combinations: CD79a/CD4/δ chain, CD8/CD4/δ chain, CD3/CD4, and CD8/CD4/perforin.

3. Results

3.1. Pathology

Clinical signs of SA-MCF in infected bison included lethargy, inappetance, corneal opacity, ocular or nasal discharge, and/or mucosal erosions. Four out of six (66%) of the infected bison had developed at least one clinical sign prior to euthanasia. OvHV-2 DNA was detected by real-time PCR in all six (100%) of the infected bison prior to and following euthanasia. All control animals tested negative for MCF virus group-specific antibody and OvHV-2 DNA in PBL samples throughout the experiment. All infected bison had at least one organ with gross lesions characteristic of SA-MCF including hemorrhagic urinary cystitis, laryngitis, pharyngitis, and/or tracheitis. All infected bison had multisystemic vasculitis involving small- to medium-caliber arteries and fewer veins. Large granular and small lympho-

cytes multifocally infiltrated the vascular tunica adventitia and tunica media and often were associated with multifocal necrosis of the vascular wall (Fig. 1A). Although ice artifact in the cryosections often displaced perivascular lymphocytes, formalin-fixed, paraffin-embedded sections verified that lymphocytes were primarily associated with vascular lesions (Fig. 1A). Gross and histologic lesions were consistent with published accounts of naturally-occurring and experimental SA-MCF in bison (Schultheiss et al., 1998, 2000; O'Toole et al., 2007). No gross or histological lesions were detected in negative control bison (Fig. 1B), and lymphocytic infiltrates were not observed in the urinary bladder, kidney, or liver.

3.2. Immunophenotyping lymphocytes in vascular lesions

The antibodies listed in Table 1 were all shown to be reactive with epitopes on bison leukocyte differentiation molecules. Immunofluorescence studies labeling single leukocyte differentiation molecules and the endothelial cell marker identified cells positive for CD79a (B cells), CD2, CD3, CD4, CD8, CD25, or the δ chain within vascular lesions of the urinary bladder in two bison. NK cells (NKp46$^+$), macrophages (M-M7$^+$ or calprotectin$^+$), and WC1$^+$ γδ T cells were only rarely identified. The detected immune cells were primarily located within the tunica adventitia and less frequently within the tunica media.

Polychromatic immunofluorescence studies concurrently identifying up to three lymphoid markers defined the proportion and phenotype of lymphocytes in vascular lesions. Findings from multiple sections of the urinary bladder, liver, and kidney of all examined bison with SA-MCF are summarized in Tables 2 and 3. Appropriate isotype controls run with each assay were negative (Fig. 2D).

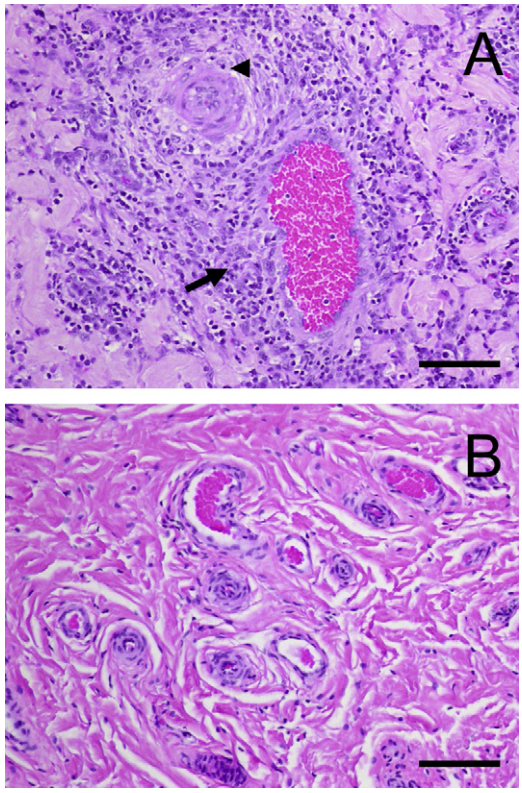

Fig. 1. Formalin-fixed, paraffin-embedded, histological sections of urinary bladder propria. The sections were stained with hematoxylin & eosin. The bars = 100 μm. (A) The section was obtained from bison 1353, a bison with SA-MCF. Lymphocytes primarily infiltrate the tunica adventitia (arrow) and tunica muscularis (arrowhead) of small to medium-caliber vessels. (B) The section was obtained from bison 1308, an uninfected control animal. There is no evidence of lymphocytic vasculitis.

Table 2
Summary of findings from polychromatic immunofluorescence studies of vasculitis lesions in the urinary bladder, kidney, and liver from all examined bison with SA-MCF. Proportions are calculated as the percentage of the total cells that labeled with either CD79a (B cells), CD4, or δ chain (γδ T cells). B cells, CD4 cells, and γδ T cells infiltrate vascular lesions in bison with SA-MCF.

Primary marker	Mean	Standard deviation	Median	Range per image	Number of images	Number of cells counted
δ chain	35.6%	15.0%	32.7%	14.0–71.3%	17	1475
CD4	51.5%	17.0%	50.7%	13.4–72.1%	17	1475
CD79a	12.9%	10.2%	9.8%	0.0–36.0%	17	1475

Table 3
Summary of co-labeling findings from polychromatic immunofluorescence studies of vasculitis lesions in the urinary bladder, kidney, and liver from all examined bison with SA-MCF. CD8$^+$perforin$^+$ γδ T cells are the predominant CD8$^+$ lymphocyte within vascular lesions ($p < 0.001$; Mann–Whitney test). CD4$^+$ cells do not express cytoplasmic perforin. The majority of CD4$^+$ cells are CD4$^+$CD3$^+$ cells ($p < 0.0001$; Mann–Whitney test), and the remainder is likely macrophages. Proportions are calculated as the percentage of the total cells that labeled with the primary immune cell marker.

Primary marker	Secondary marker	Co-labeling mean	Standard deviation	Co-labeling median	Range per image	Number of images	Number of cells counted
CD8$^+$	δ chain$^+$	95.3%	7.2%	98.6%	77.8–100.0%	10	740
	δ chain$^-$	4.7%	7.2%	1.4%	0.0–22.2%	10	740
	Perforin$^+$	98.5%	3.6%	100.0%	86.4–100.0%	16	1060
δ chain$^+$	CD8$^-$	4.6%	5.0%	2.1%	0.0–12.5%	10	740
CD4$^+$	Perforin$^+$	0.0%	0.0%	0.0%	0.0–0.0%	16	1060
	CD3$^+$	90.6%	18.0%	100.0%	5.9–100.0%	52	4449
	CD3-	9.4%	18.0%	0.0%	0.0–94.1%	52	4449
	δ chain$^+$	0.0%	0.0%	0.0%	0.0–0.0%	10	740
	CD8$^+$	0.0%	0.0%	0.0%	0.0–0.0%	10	740

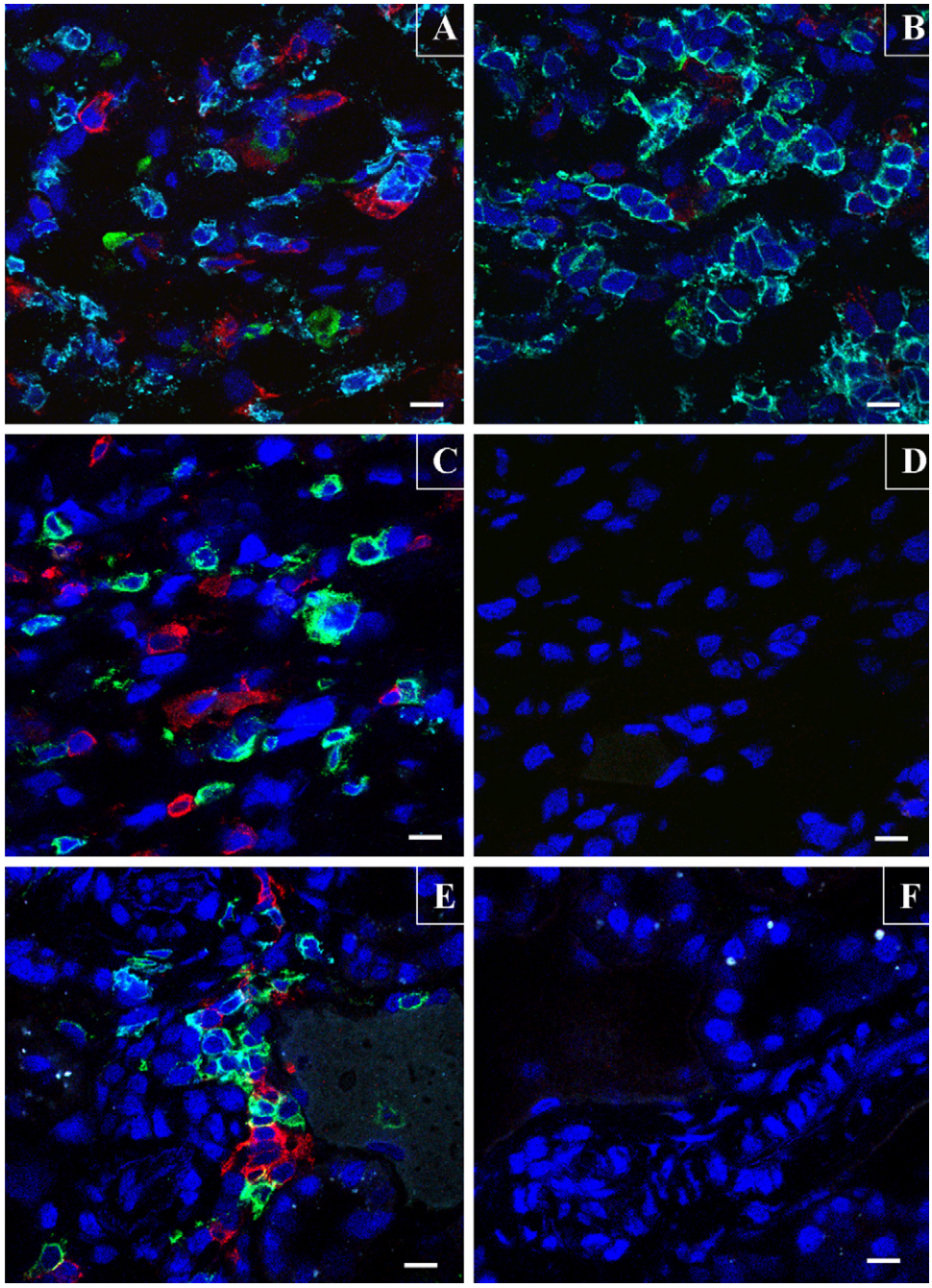

Fig. 2. Indirect polychromatic immunofluorescence on vascular lesions in ethanol-fixed, 5-μm cryosections of urinary bladder and kidney from bison with SA-MCF. Bar = 10 μm. (A) Urinary bladder of bison 1353. Four-color assay detecting DAPI (deep blue), CD79a (light green), CD4 (red), and δ chain (light blue). γδ T cells, $CD4^+$ cells, and B cells ($CD79a^+$) predominated within vascular lesions. (B) Urinary bladder of bison 1353. Four-color assay detecting DAPI (deep blue), CD8 (light green), CD4 (red), and δ chain (light blue). $CD8^+$/δ $chain^+$ cells are blue-green. $CD4^+$ does not colabel with CD8 or δ chain. (C) Urinary bladder of bison 1353. Four-color assay detecting DAPI (deep blue), CD8 (light green), CD4 (red), and perforin (light blue). Cells with cytoplasmic perforin also label with CD8 (blue-green) and not with CD4 (red). (D) Urinary bladder of bison 1353. Four-color isotype control assay detecting DAPI (deep blue) and isotype control antibodies AV64A (light green), AV213A (red), and GDBMB4 (light blue). (E) Kidney of bison 1273. Four-color assay detecting DAPI (deep blue), CD8 (light green), CD4 (red), and δ chain (light blue). $CD8^+$/δ $chain^+$ cells are blue-green. $CD4^+$ does not colabel with CD8 or δ chain. (F) Urinary bladder of bison 1353. Four-color isotype control assay detecting DAPI (deep blue) and isotype control antibodies AV64A (light green), AV213A (red), and GDBMB4 (light blue). (For interpretation of the references to color in the figure caption, the reader is referred to the web version of the article.)

Polychromatic assays concurrently labeling CD79a, CD4, and the δ chain (Table 2 and Fig. 2A) were utilized to determine the proportion of these lymphocytic phenotypes. These results showed that γδ T cells, $CD4^+$ cells, and B cells ($CD79a^+$) were present within vascular lesions at 35.6% (±15.0% sd), 51.5% (±17.0% sd), and 12.9% (±10.2% sd) of the total labeled cells, respectively.

Polychromatic studies identifying lymphoid marker co-localization on individual cells further defined the frequency and phenotype of lymphocytes in vascular lesions (Table 3). Assays concurrently labeling CD8, CD4, and the δ chain (Fig. 2B and E) showed that CD8 and δ chain markers co-localized on 95.3% (±7.2% sd) of the total cells labeling with $CD8^+$. Co-localization of CD8 and δ chain is consistent with $CD8^+$ γδ T cells. No $CD4^+$ cells expressed either CD8 or the δ chain. Assays concurrently labeling CD3 and CD4 showed that 90.6% (±18.0% sd) of $CD4^+$ cells also expressed CD3, a pan-T cell marker. Since none of the $CD4^+CD3^+$ cells expressed δ chain, these cells are consistent with CD4 αβ T cells. Assays concurrently labeling CD8, CD4, and cytoplasmic perforin (Fig. 2C) determined that perforin co-localized with 98.5% (±3.6% sd) of the cells expressing CD8 indicating a cytotoxic phenotype. Perforin did not co-localize with CD4. Therefore, $CD8^+$/perforin$^+$ γδ T cells, $CD4^+$/perforin$^-$ αβ T cells, and B cells infiltrated vascular lesions in multiple tissues of all six bison with SA-MCF.

4. Discussion

The demonstration of $CD8^+$/perforin$^+$ γδ T cells within the vascular lesions of six bison with SA-MCF supports our hypothesis that the previously identified $CD8^+$ lymphocytes are predominantly cytotoxic lymphocytes of the innate immune system. In 1994, Nakajima et al. determined that WC1 and WC2 antigens were not frequently expressed on lymphocytes in the vascular lesions of two cattle with SA-MCF. In addition, cells expressing the WC markers did not increase in either vascular or epithelial lesions as the disease progressed. Based on those observations, the authors concluded that γδ T cells likely did not play a significant role in the pathogenesis of disease. However, it is now known that a subset of γδ T lymphocytes in cattle express $CD8^+$ and do not express either WC1 or WC2 (Davis et al., 1996). Therefore, identification of the γδ T receptor is necessary in order to identify all the subsets of bovid γδ T lymphocytes. Consequently, the findings of our polychromatic immunolabeling studies redefine but do not contradict the findings of previous monochromatic studies. Since $CD8^+$/perforin$^+$ γδ T lymphocytes were consistently and frequently identified within the vascular lesions in multiple tissues of all six bison with SA-MCF, these innate immune cells may play a significant role in the pathogenesis of SA-MCF in bison.

There are many possible ways that $CD8^+$/perforin$^+$ γδ T lymphocytes might contribute to the pathogenesis of vasculitis in bison with SA-MCF. Human and bovine γδ T cells can be activated by pathogen-associated molecular patterns (PAMPs) (Hedges et al., 2005), and, as a result, have greatly increased proliferative responses to IL-2 or IL-15 (Jutila et al., 2008). Interestingly, abundant IL-15 expression has been observed in tissues of rabbits with SA-MCF, and large granular lymphocytes obtained from the tissues of rabbits infected with OvHV-2 exhibited a similar proliferative response to IL-15 or IL-2 in culture (Anderson et al., 2008). $CD4^-$/$CD8^-$ T cells have been identified as the lymphoproliferative component in rabbits with experimentally-induced SA-MCF (Anderson et al., 2007), and rabbit γδ T lymphocytes do not express CD4 or CD8 (Jeklova et al., 2007). Consequently, γδ T lymphocytes may play an important role in the pathogenesis of vasculitis in both rabbits and bison with SA-MCF.

In our study, expression of detectable cytoplasmic perforin implies the γδ T cells are activated for cytotoxicity, and it is possible that vascular necrosis could be mediated by these cells. Furthermore, there is evidence that bovine $CD8^+$ γδ T cells selectively migrate to mucosal tissues (Wilson et al., 2002), and these cells could be recruited to sites of inflammation. As cytotoxic effector cells with MHC-unrestricted cytotoxicity, γδ T cells could mediate necrosis of vascular structural cells via antibody-dependent cytotoxicity as suggested in the pathogenesis of multiple sclerosis (Chen and Freedman, 2008), or kill virally infected target cells as observed with human infections with cytomegalovirus, a betaherpesvirus (Halary et al., 2005). The γδ T cells in the vasculitis lesions of bison with SA-MCF may also be serving an immunoregulatory function since γδ T cells have regulatory function in humans and cattle (Meissner et al., 2003). Further characterization of the $CD8^+$ γδ T cells within the vascular lesions will require evaluation of their function and cytokine expression profiles.

The $CD4^+$/perforin$^-$ αβ T cells presumably play a regulatory role in the pathogenesis of vasculitis in bison with SA-MCF. Further studies will be needed to determine whether these cells function as T regulatory cells or T helper cells including Th1, Th2, or Th17 cells. However, a cytotoxic role for these $CD4^+$ cells cannot be completely excluded since T regulatory cells in mice can induce cell death in target lymphocytes via Fas–Fas ligand interactions (Marks, 2004).

$CD3^-$/$CD4^+$ cells were infrequently and inconsistently identified within vascular lesions (see Table 3). CD4 is expressed on some human and rat macrophage subsets (Crocker et al., 1987), and this finding has also been observed in cattle (personal communication, W.C. Davis). Cells expressing DH59B, a monocyte/macrophage marker, have been previously identified in the vascular lesions of two cattle (Nakajima et al., 1994) and one bison (Simon et al., 2003) with SA-MCF. Consequently, these $CD3^-CD4^+$ cells are likely macrophages.

Though B cells do not appear to play a direct role in the pathogenesis of vasculitis, γδ T cells mediate antibody-dependent cytotoxicity in multiple sclerosis (Chen and Freedman, 2008), and antibody may therefore play a role in the pathogenesis of SA-MCF. Furthermore, there is no current evidence of B cell infection in cattle or bison with SA-MCF. However, it is possible that viral tropism for specific lymphocytes may vary among ruminant species. Further investigation is needed to determine the importance of B cells in sheep and clinically susceptible species.

In summary, $CD8^+$ γδ T cells and $CD4^+$ αβ T cells both have potential for cytotoxicity and regulatory function, and both may therefore contribute to the pathogenesis

of vasculitis. Since there is evidence of OvHV-2 infection of $CD8^+$ lymphocytes and no current evidence of infection of cells that compose the vascular wall (Simon et al., 2003), we propose that infection of lymphocytes causes immune dysregulation and immune-mediated vasculitis in bison with SA-MCF. Since bison are particularly susceptible to developing fatal disease, SA-MCF is considered one of the most important threats to wild and domestic bison herds. This disease also complicates management programs for endangered species in dense populations such as animal parks, zoos, or preserves. Improving understanding of immunopathological mechanisms of disease in SA-MCF will assist in developing appropriate vaccine strategies to prevent disease in susceptible animals.

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